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Chinese Chemical Letters 22 (2011) 1443–1446
CHINESE CHEMICAL LETTERS
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Solution-phase synthesis of a muramyl dipeptide analogue MDA

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Received 25 May 2011
Available online 10 October 2011

Abstract

The solution-phase synthesis of a muramyl dipeptide (MDP) analogue of N^{α}-[4-chlorocinnamoyl-L-alanyl-D-isoglutaminyl]-L-lysine (MDA, **2**) is reported that possesses the features of easy feasibility, safety and low cost in large scale of synthesis.

Keywords: Muramyl dipeptide analogue; Solution-phase synthesis; MDA

Muramyl dipeptide (MDP, **1**, Fig. 1) is the minimum structure of the cell wall of Gram-positive bacteria that is recognized by human immunological response. It non-specifically stimulates human macrophages to become active against virus, bacteria, and tumors [1–3]. In view of its activities, we embarked on the structural modification of MDP for many years [4–8]. A MDP analogue of N^{α}-[4-chlorocinnamoyl-L-alanyl-D-isoglutaminyl]-L-lysine (MDA, **2**, Fig. 1) has been conjugated with Paclitaxel in our group to synthesize a novel compound termed as MTC-220 [8]. The unique pharmacological property allows MTC-220 to effectively inhibit tumor growth in nude mice and significantly prevent tumor metastasis in Lewis lung carcinoma and 4T1-tumor-bearing mice through suppressing myeloid derived suppressor cell (MDSC) accumulation in the spleen and bone marrow of tumor-bearing mice and repressing inflammatory cytokines in tumor tissue [8]. MTC-220 is currently under research for its preclinical studies as a dual function drug candidate to treat breast and lung cancers. Therefore, it is important to find a feasible method for production of MTC-220 as well as its intermediate MDA in large scale.

We previously reported that MDA was prepared through a solid-phase synthetic route with the total yield of 96% [8]. Solid-phase synthesis is a common strategy of peptide chemistry. The advantages of this method mainly include highly coupling efficiency and yield. However, drawbacks of solid-phase synthesis often limit the preparation of aim product in large scale including the necessary and expensive solid resin, costly washing and cleavage solvents, incomplete/truncated peptides and/or resin anchored impurities that are produced in the process of synthesis. Especially, the building blocks of non-natural amino acids need to be pre-prepared and pre-protected in solution-phase where addresses additional chemical processes. Therefore, alternative solution-phase synthesis of certain peptide or peptide analogue, especially bearing non-natural amino acids, is normally considered to facilitate the production in

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1001-8417/$ – see front matter
doi:10.1016/j.cclet.2011.07.020

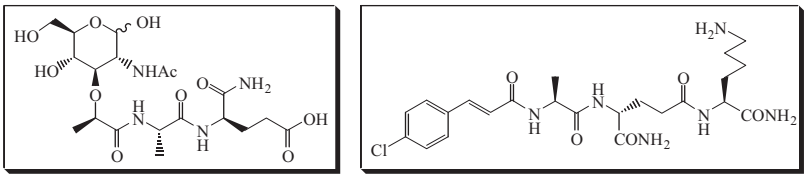

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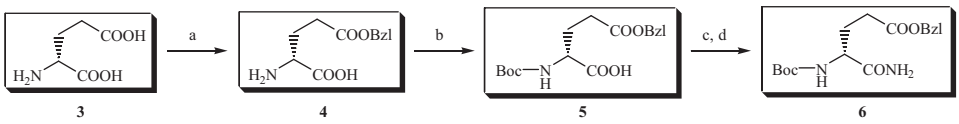
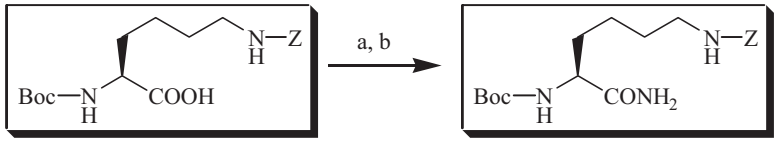
Fig. 1. Chemical structure of the natural MDP **1** and its analogue MDA **2**.



3 4 5 6

Scheme 1. Synthetic route of *t*-butoxycarbonyl-D-isoglutaminyl benzyl ester **6**. Reagents and conditions: (a) benzyl alcohol, $BF_3{\cdot}Et_2O$, r.t., 15 h; (b) $(Boc)_2O$, $NaHCO_3$, 50% dioxane/H_2O, r.t., 20 h; (c) HOSu, DIC, 0 °C, 5 h, r.t., 20 h; (d) dry ammonia gas, THF, −10 °C, 1.5 h.



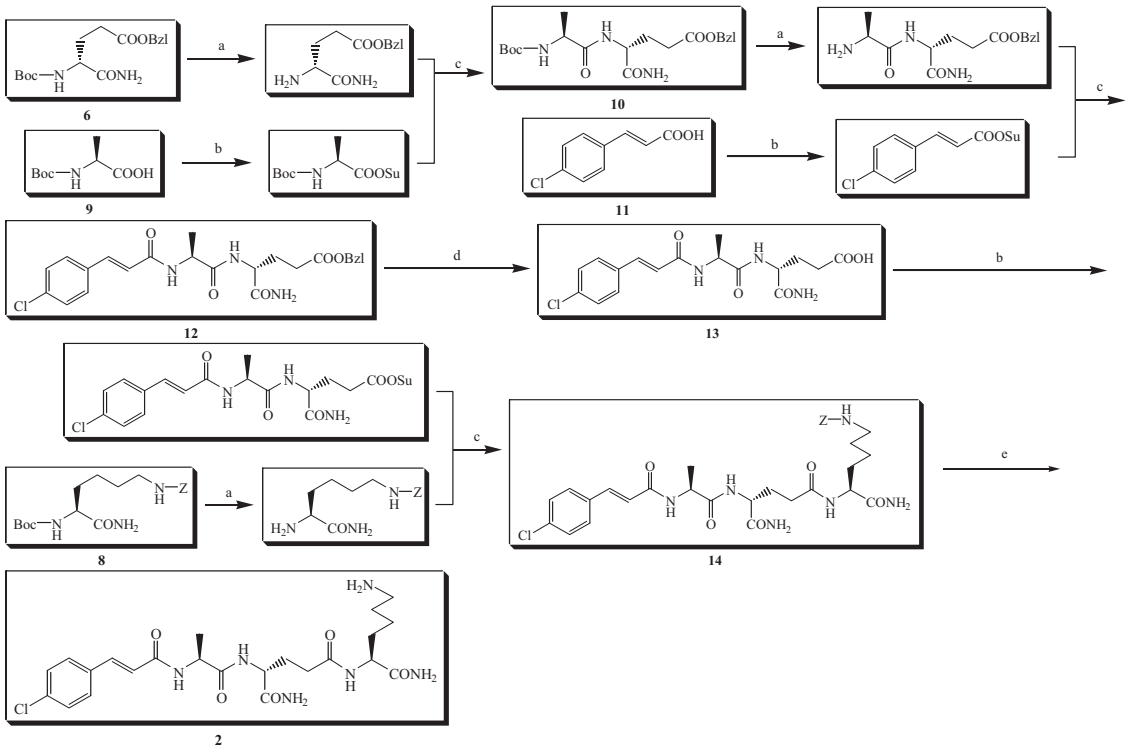
7 8

Scheme 2. Synthetic route of N^{α}-*t*-butoxycarbonyl-N^{ε}-carbobenzyloxy-L-lysinyl amide **8**. Reagents and conditions: (a) HOSu, DIC, 0 °C, 5 h, r.t., 20 h; (b) dry ammonia gas, THF, −10 °C, 1.5 h.

large scale. In this letter, we reported a solution-phase synthetic method of MDA that can be used to prepare MDA in kilogram scale (Schemes 1–3).

t-Butoxycarbonyl-D-isoglutaminyl benzyl ester **6** and N^{α}-*t*-butoxycarbonyl-N^{ε}-carbobenzyloxy-L-lysinyl amide **8** were prepared from commercial available D-glutamic acid **3** and N^{α}-*t*-butoxycarbonyl-N^{ε}-carbobenzyloxy-L-lysine **7** in Schemes 1 and 2, respectively. γ-Carboxyl group of **3** was firstly esterified in benzyl alcohol to gain D-glutaminyl γ-benzyl ester **4** in the presence of Lewis acid $BF_3{\cdot}Et_2O$ [9]. The amino group of **4** was then protected by $(Boc)_2O$ to yield *t*-butoxycarbonyl-D-glutaminyl γ-benzyl ester **5** under a weakly basic condition [10]. Compound **5** was further activated by HOSu and DIC, then reacted with dry ammonia gas to finally form compound **6** in a yield of 53% (Scheme 1) [4,5]. Similarly, compound **8** was obtained by the method in a yield of 92% shown in Scheme 2.

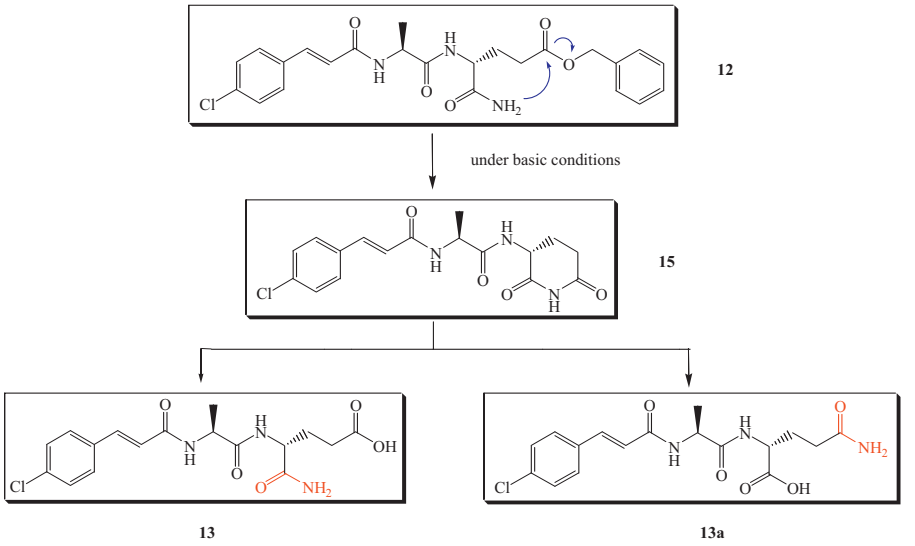
The peptide assembly in solution-phase was carried out as described in Scheme 3. All Boc groups in various building blocks were generally removed under an acidic condition of 50% TFA/DCM (v/v). And all coupling steps were performed by HOSu and DIC activation of carboxyl groups. Building blocks of *t*-butoxycarbonyl-L-alanine **9** and *t*-butoxycarbonyl-D-isoglutaminyl benzyl ester **6** were firstly coupled to yield a protected dipeptide of *t*-butoxycarbonyl-L-alaninyl-D-isoglutaminyl benzyl ester **10** in 81% yield. After removal of Boc group and performance of coupling reaction again, a protected tripeptide of 4-chlorocinnamoyl-L-alanyl-D-isoglutaminyl benzyl ester **12** was obtained in a total yield 79% of two reaction steps. Considering the sensitivity of chlorine atom and a double bond of 4-chlorocinnamoyl group, palladium–charcoal/hydrogenation was avoided to deprotect Bzl group in this letter. Various basic conditions were investigated to completely cleave Bzl group such as K_2CO_3, $NaHCO_3$ and LiOH aqueous solution. Unexpectedly, two major products (molar ratio = 4:6) with the same molecular weight were gained at all the time. Results of elucidation by ^1H NMR indicated that they were the aim compound 4-chlorocinnamoyl-L-alanyl-D-isoglutamine **13** and its structural isomer 4-chlorocinnamoyl-L-alanyl-D-glutamine **13a** that very likely were produced through a cyclic intermediate 3(R)-[4-chlorocinnamoyl-L-alanyl]-amino-2,6-dioxopiperidine **15** (Scheme 4). We then switch our attention to acidic conditions to deprotect Bzl group. A solution of HBr/AcOH was optimally selected to afford **9** in a high yield of 85%. Final iteration of the deprotection of Boc group and coupling procedure led N^{α}-[4-chlorocinnamoyl-L-alanyl-D-isoglutaminyl]-N^{ε}-carbobenzyloxy-L-lysine **14** produced in a yield of 74%. With the assistance of a mixture of $BF_3{\cdot}Et_2O$, TFA and EtSH (v:v:v = 9:9:2), Z



Scheme 3. Solution-phase synthesis of MDA. Reagents and conditions: (a) 50% TFA/DCM, r.t., 1 h; (b) HOSu, DIC, THF, 0 °C, 5 h, then, r.t., 20 h; (c) THF, 0 °C, 5 h, r.t., 24 h; (d) HBr/AcOH, r.t., 3 h; (e) $BF_3{\cdot}Et_2O$, TFA, EtSH (9:9:2), r.t., 2 h.

group of **14** was completely removed with a yield of 72%. The aimed MDA **2** was obtained eventually after purification by ODS column chromatography and fully characterized by ^{1}H NMR, ^{13}C NMR, IR and HR MS (TOF) [11].

In conclusion, we have developed a solution-phase synthesis of MDA. This strategy can be employed to synthesize a novel antitumor agent of MTC-220 in kilogram scale. After all steps, targeted compound MDA was obtained with the total yield of 14%.



Scheme 4. Structural isomers of **13** produced under basic conditions.

Acknowledgment

This research is supported financially by the National Natural Science Foundation of China (No. 90713045).

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[11] Chemical data of (**2**) list as following: mp: 215–217 °C, $[\alpha]_D^{25}$ +37.7 (*c* 11.0 mg/mL, DMF). ^{1}H NMR (600 MHz, DMSO-d_6): δ lysine's part: 7.90 (d, 1H, *J* = 8.4 Hz, NH), 7.10 (s, 1H, $CONH_a$), 7.30 (s, 1H, $CONH_b$), 4.11 (m, 1H, α-H), 1.46 (m, 1H, β-H_a), 1.63 (m, 1H, β-H_b), 1.27 (m, 2H, γ-H), 1.53 (m, 2H, δ-H), 2.73 (m, 2H, ε-H), 7.75 (br. s, 2H, NH_2); D-isoglutamine's part: 8.21 (d, 1H, *J* = 8.4 Hz, NH), 6.98 (s, 1H, $CONH_a$), 7.41 (s, 1H, $CONH_b$), 4.14 (m, 1H, α-H), 1.71 (m, 1H, β-H_a), 1.97 (m, 1H, β-H_b), 2.15 (t, 2H, *J* = 7.2 Hz, γ-H); alanine's part: 8.39 (d, 1H, *J* = 6.6 Hz, NH), 4.38 (m, 1H, α-H), 1.26 (m, 3H, β-H); 4-chloro-cinnamoyl part: 6.75 (d, *J* = 15.9 Hz, 1H, *trans*-α-H), 7.39 (d, 1H, *J* = 15.9 Hz, *trans*-β-H), 7.57 (d, 2H, *J* = 8.4 Hz, ph-*o*-H), 7.47 (d, 2H, *J* = 8.4 Hz, ph-*m*-H). ^{13}C NMR (150 MHz, DMSO-d_6): δ lysine's part: 173.3 ($CONH_2$), 52.1 (α-C), 31.3 (β-C), 22.4 (γ-C), 26.8 (δ-C), 38.7 (ε-C); D-isoglutamine's part: 173.8 ($CONH_2$), 52.2 (α-C), 27.7 (β-C), 31.7 (γ-C), 171.6 (CONH); alanine's part: 172.4 (CONH), 48.8 (α-C), 18.1 (β-C); 4-chloro-cinnamoyl part: 164.7 (CONH), 122.7 (*trans*-α-C), 137.6 (*trans*-β-C), 133.8 (ph-*q*-C), 129.2 (ph-*o*-C), 129.0 (ph-*m*-C), 134.0 (ph-*p*-C). IR (KBr, cm^{-1}): 3281.5, 3198.9, 3063.4, 2935.2, 1609.8, 1539.0, 1452.2, 1200.2, 1134.1, 973.6, 821.6, 799.8, 720.2. HR MS (TOF): found 509.2270 $[M+H]^+$; calcd. for $C_{23}H_{34}ClN_6O_5$ 509.2279.